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A PROSPECTIVE STUDY OF BIOMARKERS LEVELS IN MANAGEMENT OF CHRONIC OSTEOMYELITIS

Orthopaedics

Dr. Shobhit Kumar Junior Resident, GSVM Medical College, Kanpur

Dr. Prof. Sanjay Kumar Head Of Department And Professor, Department Of Orthopaedics, GSVM Medical College, Kanpur

Dr. Abha Jyoti Associate Professor, Department Of Biochemistry, GSVM Medical College, Kanpur

Dr. Yogendra Narayan Verma Associate Professor, Department Of Pathology, GSVM Medical College, Kanpur

Dr. Shahid Ahmad Lari Junior Resident, GSVM Medical College, Kanpur

ABSTRACT

Chronic osteomyelitis is a debilitating disease characterized by osteonecrosis, where disrupted blood flow leads to bone fragmentation and sequestra formation. These sequestra harbor infectious agents that evade the immune system and antibiotics, causing persistent infection. The disease is marked by elevated inflammatory markers like neutrophils, plasma cells, and lymphocytes, leading to severe complications, including limb deformity and infection. This study investigates the use of osteocalcin and osteopontin as clinical markers for chronic osteomyelitis. Two patient groups, treated with medication or surgery, showed significantly higher post-treatment levels of osteocalcin, osteopontin, CRP, and ESR, suggesting their potential role in diagnosis and monitoring.

KEYWORDS

Osteocalcin, Osteopontin, Chronic Osteomyelitis, C-reactive protein, Erythrocyte sedimentation rate, Osteoblast, Bone mineral density

INTRODUCTION

An osseous inflammation linked to a microbial infection is called osteomyelitis [1]. Chronic osteomyelitis is a prevalent clinical illness that can be difficult to diagnose early due to its high rate of impairment, lengthy disease course, and difficulty. Different geographic locations, periods of time, and pathogenetic variations can all have an impact on the clinical features of chronic osteomyelitis.

Chronic osteomyelitis is a complex disease with serious repercussions. Geographically, the condition is more common in developing than in industrialised nations; this is probably due to disparities in economic status, way of life, and access to healthcare [2]. With ageing, the incidence increases and peaks in patients between the ages of 50 and 70 at 6.5 per 100,000 [3].

The number of patients suffering from multiple fractures and open wounds from industrial and traffic accidents has increased dramatically in recent years due to the economy's rapid development [4]. The infection may affect numerous areas of the skeleton, including the periosteum, cortex, marrow, and soft tissue in the vicinity, or it may just affect one section of the bone. Any bone may be affected, and it can happen at any age. Because of their heightened susceptibility to infection and treatment complications, it is imperative to identify individuals who have foreign-body implants. Several complications, including limb deformity, septic arthritis, deep vein thrombosis, pathologic fracture, sepsis, or even death, are more likely to occur when detection is delayed. Therefore, it is imperative to identify and treat the condition as soon as possible. Patients with progressive osseous degradation and sequestrum formation, indicative of chronic osteomyelitis, were commonly observed [5].

During diagnosis and follow-up, markers of this inflammatory reaction, such as erythrocyte sedimentation rate (ESR) or C-reactive protein (CRP), may be examined. However, the results of these tests are not always indicative of the continuance or worsening of a bone infection because they are non-specific. Additional data for future clinical care may be obtained from a particular biochemical marker for bone metabolism [6]. Because osteoblasts are the only cells that make osteocalcin, a non-collagenic bone protein, it indicates the rate at which bone regenerates [7]. Osteoblasts and osteoclasts involved in bone structure release OPN into the osseous tissue [8]. It is known that OPN produced by osteoclasts inhibits hydroxyapatite [10], suggesting a link between OPN and bone deterioration [9].

This investigation aimed to explore the potential utility of blood levels of osteocalcin and osteopontin as a clinical marker for individuals suffering from chronic osteomyelitis.

MATERIALS AND METHODS

Study Design

From December 2022 to July 2024, this prospective study was conducted in the Department of Orthopaedics, in collaboration with the Department of Biochemistry at GSVM, Kanpur. Institutional Ethics Committee (Observational Studies), GSVM, Kanpur,India, approved the study and before recruiting the research participants in study, they had given informed consent in writing. The research was in accordance with the 2017 ICMR National Biomedical Guidelines for Research in Human Participants.

Study Procedure

Sample size was calculated using the Power and Sample Size Calculator version 3.0, the minimum sample size needed for the study was calculated to be 125. Inclusion criteria for cases were the participants diagnosed with chronic osteomyelitis and age between 2-60 years. Participants who lost to follow up were excluded. Based on inclusion and exclusion criteria, known cases of chronic osteomyelitis were recruited from the Department of Orthopaedics at GSVM, Kanpur. Light application of a tourniquet was followed by blood sampling from the antecubital vein. 6 ml blood sample was taken after taking written informed consent. Serum was separated and stored at -80 degree C for further evaluation of biochemical variables. Osteopontin and osteocalcin were assayed by ELISA. ESR and CRP were noted down.

Statistical Analysis

The data analysis was done using both descriptive and inferential statistics. The categorical data such as age, gender, and employment status, were explained through the application of percentages and frequencies. The data were expressed as mean and standard deviation or median with interquartile range depending on the distribution of the variables (osteopontin and osteocalcin). Independent Student t-test was used to compare normally distributed continuous data, while the Mann Whitney "U" test was applied to non-Gaussian data. The variation in the parameters within the groups will be analyzed using paired t test/Wilcoxon signed rank sum test. To investigate the relationship between the variables, Spearman correlation analysis was used. In order to conduct the statistical analysis, SPSS programme 20 (SPSS Inc) was used. p-value <0.05 was regarded as statistically significant.

RESULTS

Table 1 gives the participant's characteristics in Group 1 (managed by

medicine) and the group-2 (managed by surgery). The mean age (in years) of the study participants in group-1 and group-2 were 24 (11.5-38) and 26 (17-40) respectively. The number of females and males in group 1 was 17 & 37 respectively. The number of females and males in group 2 was 23 & 48 respectively. In group 1, 21 were employed and 33 were unemployed. In group 2, 36 were employed and 35 were unemployed. From Table 1 and Figure 2 in both the groups, Staphylococcus aureus was isolated a maximum no. of times in culture i.e. 39 participants from group-1 and 46 participants from group 2 were infected with Staphylococcus aureus. In Table 1, a maximum number of participants had no co-morbidity. Out of 54 in group 1, 41 had no co-morbidity, 3 were hypertensive, 9 were diabetic, 1 was autoimmune and from group 2, out of 71, 54 had no co-morbidity, 11 were hypertensive, 3 were diabetic and 3 had both hypertension & diabetes. In group 1, 46 participants were identified with sinuses and 8 had no sinuses. In group 2, 68 participants were identified with sinuses and 3 had no sinuses. In table 2, inflammatory indices (ESR and CRP) were compared between group-1 and group-2. The ESR and CRP of the participants in the two groups did not significantly differ from one another according to our research. Table 3 shows the comparison of pre & post-data between participants of group-1 (medical management) & group-2 (surgical management). Pre-management & post-management parameters in both groups were found to be similar. Table 4 compares pre-management & post-management osteocalcin and osteopontin levels among the two groups. In both the groups, post-management osteocalcin levels were determined to be noticeably greater than pre-management osteocalcin levels. Similar trend was observed in osteopontin levels also in both groups with relative higher increment in group 2. Increment in osteocalcin was found relatively higher when comparing group 1 to group 2. Most of the afflicted bones in chronic osteomyelitis are seen in Figure 4. We discovered that the most often impacted bone in chronic osteomyelitis is the tibia. Figure 5 illustrates that 26% of the upper limbs and 74% of the lower extremities are afflicted with chronic osteomyelitis. Figure 6 illustrates the sex distribution among two groups. Figure 7 shows clinico-radiological image of chronic osteomyelitis of both bone leg. Figure 8 shows clinico-radiological image of chronic osteomyelitis of distal 3rd femur. Figure 9 shows clinic-radiological image of midshaft tibia right side.

Table 1: Participant's characteristics by study groups

Variables	Group-1 (Medical management) N=54	Group-2 (Surgical management) N=71
Age (years)	24 (11.50-38.00)	26 (17.00-40.00)
Gender		
Female	17 (31.48%)	23 (32.39%)
Male	37 (68.51%)	48 (67.60%)
Occupational status		
Employed	21 (38.88%)	36 (50.70%)
Unemployed	33 (61.11%)	35 (49.29%)
Culture & organism isolated		
Staphylococcus aureus	39 (72.22%)	46 (64.78%)
Salmonella	5 (9.25%)	10 (14.08%)
Group B Streptococcus	5 (9.25%)	6 (8.45%)
Pseudomonas aeruginosa	2 (3.70%)	4 (5.63%)
E. coli	2 (3.70%)	3 (4.22%)
Proteus	-	2 (2.81%)
Enterococci	1 (1.85%)	-
Co-morbidity		
No co-morbidity	41 (75.92%)	54 (76.05%)
Hypertensive	3 (5.55%)	11 (15.49%)
Diabetes	9 (16.66%)	3 (4.22%)
Diabetes & Hypertensive	-	3 (4.22%)
Autoimmune	1 (1.85%)	-
Sinus		
Present	46 (85.18%)	68 (95.77%)
Absent	8 (14.81%)	3 (4.22%)

Table 2: Comparison of routine parameters between participants managed by medical management and surgical management

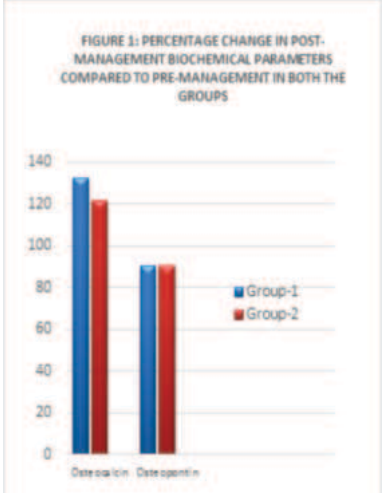
Variables	Group-1 (Medical management) (N=54) Median (IQR)	Group-2 (Surgical management) (N=71) Median (IQR)	*p-value
ESR (mm/hr)	25 (20-28)	25 (21-29)	0.637
CRP (mg/dl)	12.50 (10-18)	13 (10-17)	0.481

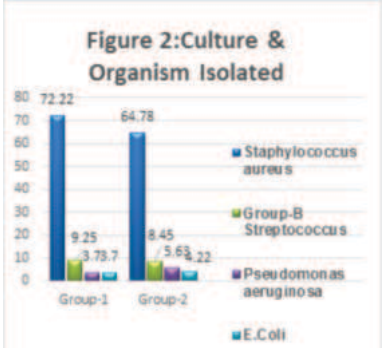
Table 3: Comparison of pre and post data between participants of group-1 (medical management) & group-2 (surgical management)

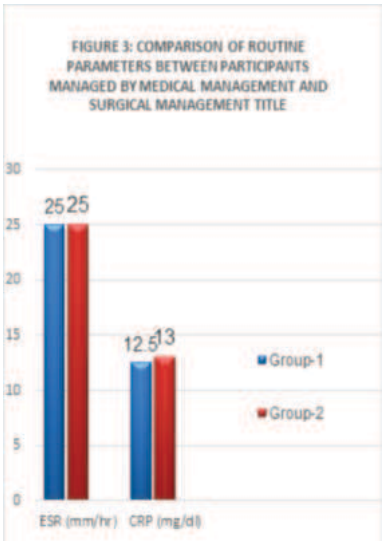
Variables	Group-1 (Medical management) (N=54) Median (IQR)	Group-2 (Surgical management) (N=71) Median (IQR)	*p-value
Osteocalcin			
Pre-management osteocalcin (ng/ml)	14.00 (10.00-18.00)	14.00 (11.00-18.00)	0.581
Post-management osteocalcin (ng/ml)	32.50 (27.75-38.00)	31.00 (19.00-37.00)	0.181
Osteopontin			
Pre-management osteopontin (ng/ml)	10.00 (8.00-10.25)	10.00 (7.00-12.00)	0.790
Post-management osteopontin (ng/ml)	19.00 (12.75-28.00)	19.00 (14.00-27.00)	0.861

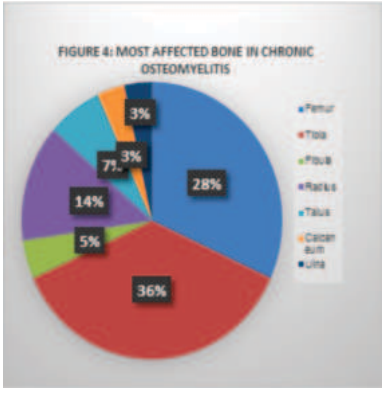
Table 4: Comparison of pre- management & post- management osteocalcin and osteopontin among group-1 (medical management) and group-2 (surgical management)

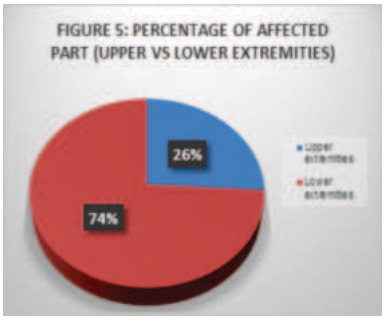
Variables	Group-1 (Medical management) (N=54) Median (IQR)			Group-2 (Surgical management) (N=71) Median (IQR)		
	Pre-manage-ment	Post-manage-ment	*p-value	Pre-manage-ment	Post-manage-ment	*p-value
Osteocalcin (ng/ml)	14.00 (10.00-18.00)	32.50 (27.75-38.00)	0.00	14.00 (11.00-18.00)	31.00 (19.00-37.00)	0.00
Osteopontin (ng/ml)	10.00 (8.00-10.25)	19.00 (12.75-28.00)	0.00	10.00 (7.00-12.00)	19.00 (14.00-27.00)	0.00











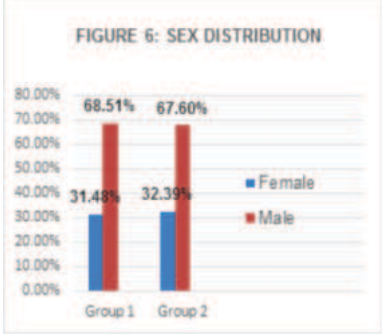


Figure 7: Clinico-radiological Image Of Chronic Osteomyelitis Of Both Bone Leg

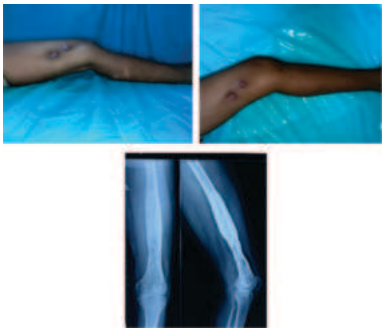


Figure 8: Clinico-radiological Image Of Chronic Osteomyelitis Of Distal 3rd Femur

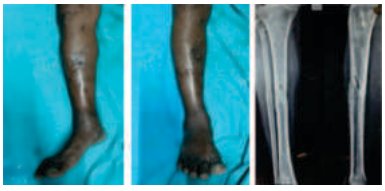


Figure 9: Clinico-radiological Image Of Midshaft Tibia Right Side

DISCUSSION

Treatment for chronic osteomyelitis, which can last for weeks, months, or years, and is characterized by recurrent episodes of low-level inflammation, dead bone, fresh osseous implantation, and fistulous tracts, involves long-term broad-spectrum antibiotics and potentially multiple surgical procedures [11, 20]. We discovered that the age range in which the greatest number of patients with chronic osteomyelitis presented was between 11.5 and 38 years old in group 1 (treated with medication) and 17 to 40 years old in group 2 (treated with surgery). Our study revealed that men were more affected than women. As men are more prone to participate in physically demanding jobs or high-risk activities, this is consistent with Kremers's findings, which suggested that an increase in industrial and traffic accidents was a factor in the higher number of male patients [3, 21, 22]. Our goal in this study was to compare the variations in pre & post-management levels of osteocalcin and osteopontin among patients receiving medical or surgical treatment. The majority of patients in our study did not have co-morbidity in both categories. In both groups, the majority of subjects were diagnosed with sinus. In both categories, the percentage of unemployed patients were found to be slightly higher. A critical first step in the management of chronic osteomyelitis is identifying the pathogenic microorganisms involved. Effective antibiotics that are unique to the pathogenic bacteria should be used in clinical practice based on their features; this is a prerequisite to ensuring a therapeutic effect. The rate at which harmful bacteria were detected could be increased by sending multiple samples at once. Staphylococcus aureus is the most frequent bacterium involved in all processes that lead to osteomyelitis. Methicillin-resistant S. aureus (MRSA) is the organism that causes a significant amount of antibiotic-resistant infections [12, 23]. It is evident from our data that Staphylococcus aureus was the predominant cause of the chronic osteomyelitis in most of the patients. Most of the times, Staphylococcus aureus was isolated from the culture in each group. One possible traumatic cause for traumatic osteomyelitis is traffic trauma, a high energy trauma that frequently results in an open wound. Furthermore, this age group has a high rate of diabetes, which raises the risk of osteomyelitis associated with diabetes [3, 25]. The thin soft tissue around the tibia and lack of blood supply can increase the risk of wound infection, which is followed by chronic osteomyelitis. As mentioned above, such accidents are more likely to result in trauma with an open wound. Therefore, the most common type of osteomyelitis occurs in the tibia [14]. We found the most often affected areas in chronic osteomyelitis were the lower limbs. Bacteria can travel throughout a long bone via the Haversian and Volkmann canal networks, local vasculature, and other pathways. Reduced blood flow and elevated intramedullary pressure are the outcomes of the first immune response-induced inflammation. Additionally, S. aureus causes bone thrombosis by producing coagulases, which when combined with localized inflammation, cause bone necrosis [14,24]. CRP was the acute phase reactant that in the current study demonstrated the strongest correlation with the infection's clinical activity [15, 26]. We found that the majority of patients had CRP levels greater than 5 mg/dl and an ESR greater than 20 mm/hr [1]. The

levels of inflammatory markers (CRP & ESR) showed no discernible difference between the two groups, according to our findings. In all diseases where bone metabolism is increased, osteocalcin is elevated. Osteoblast activation causes increased levels of both primary and secondary hyperparathyroidism [16]. Our findings showed a substantial variation in the pre- and post-management levels of osteocalcin and osteopontin in each group. However, we were unable to draw any conclusions about the relative efficacy of medical and surgical treatment since we were unable to detect any discernible variations in the concentrations of osteopontin & osteocalcin among the patients receiving medical management and those receiving surgical treatment. Increased osteocalcin levels were observed in osteoporosis [17, 27, 28, 29]. When these patients were first started on medication, there was no discernible relationship between osteocalcin levels and inflammatory activity. However, following treatment with penicillamine and chloroquine, osteocalcin levels increased concurrently with a decrease in acute-phase proteins [18]. Serum osteocalcin levels did not significantly change in response to an increase in inflammatory activity in patients with chronic osteomyelitis [6]. The proinflammatory state and the function of OPN in immunological disorders. OPN is primarily secreted by osteoblasts and is thought to make up 2% of non-collagenous proteins in bone tissue. Research revealed inverse relationships between BMD in the lumbar spine and serum OPN levels [19, 30]. Our analysis supports the idea that serum osteopontin & osteocalcin levels might be used for the early and improved detection of chronic osteomyelitis. This analysis gives valuable clinical information for the management of patients treated for chronic osteomyelitis since it shows that there are quantifiable changes in the serum levels of osteocalcin and osteopontin. When treating chronic osteomyelitis, it is important to carefully assess the patient's state and make thoughtful treatment decisions by taking into account both the local lesion and the patient's general health. The clinical literature offers several treatments for chronic osteomyelitis, including bone transport, acute limb shortening, radical debridement, two-staged reconstruction, intramedullary nails, spacers, antibiotic-impregnated beads, and cements; and soft tissue grafting (free flap, myocutaneous flap, skin graft). Nevertheless, there isn't yet a set standard for diagnosis or care. There are now two main agreements on how to treat persistent osteomyelitis. First, every treatment depends on debridement. Furthermore, determining which pathogenic bacteria are present and using the right antibiotics are necessary to ensure a therapeutic impact. PMMA spacers loaded with local antibiotics ought to be used to treat the bone defect location. One can stabilise fracture ends via internal or external fixation.

CONCLUSION

This study confirms a possible connection between chronic osteomyelitis and the serum biomarkers- osteocalcin and osteopontin. Post-management osteocalcin and osteopontin levels were greater in all groups when compared to pre-management osteocalcin and osteopontin levels in the serum. Osteopontin and osteocalcin levels, both before and after treatment, are within the normal range. Post-management values are higher than pre-management values, as we have observed. Thus, we propose that it represents the body's defense mechanism against chronic osteomyelitis. It is utmost importance to identify and treat chronic osteomyelitis in these participants, as inflammation is a part of pathways driving necrosis of bone. This could be the basis of future research in the field of medicine as an adjunct to standard treatment to see if it could ameliorate the chronic osteomyelitis. There were various limitations to the current investigation. Given that it was carried out at a single medical facility, it might not accurately represent chronic osteomyelitis throughout the region. As a result, multicenter research ought to be carried out to get more comprehensive information. The majority of patients got oral antibiotics outside of hospitals, which meant that this study lacked comprehensive statistical data on oral antibiotic therapy. We attempted to differentiate between the effectiveness of modes of treatment but we couldn't find it due to unequal number of participants in each group.

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